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Pharmacological intervention

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An abbreviated version of this protocol was published in Science Advances in Mar 2021

IL4-driven microglia modulate stress resilience through BDNF-dependent neurogenesis

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Detailed protocol

Isolation of microglia from the hippocampus

The mice were decapitated, their brains were separated and placed in PBS in an ice bath. Bilateral hippocampal tissue was separated from the cerebral hemispheres. Hippocampi from 8-14 mice were collected in a sterile centrifuge tube and homogenized in 1x phosphate buffered saline (PBS, pH 7.4) by passing through a 70 μm nylon cell strainer. Resulting homogenates were centrifuged at 600 × g for 6 min. Supernatants were removed and cell pellets were re-suspended in 70% isotonic Percoll at room temperature. A discontinuous Percoll density gradient was layered as follows: 70%, 50%, 35%, and 0% isotonic Percoll. The gradient was centrifuged for 20 minutes at 2000 × g and microglia were collected from the interface between the 70% and 50% Percoll layers. Microglia were washed and re-suspended in sterile filtered PBS or FACS buffer. Each group extraction yielded approximately 3×10^5 viable cells. These cells as approximately 90% $CD11b^+/CD45^{low}$ microglia.

NSPCs culture

Neural stem/progenitor cells (NSPCs) were generated from the subependymal ventricular zone (SVZ) of young adult mice and cultured under clonal conditions. Briefly, for each culture, the entire SVZ region was dissected from sagittally cut brains and incubated in DMEM containing 20 U/ml papain (Worthington), 1 mM N-acetyl-l-cysteine (NAC; Sigma) and 2 U/ml RQ1 DNase (Promega) for 1 h at 37 °C. Dissociated cells were plated in 24-well plates at a density of 2.6×10^3 cells/cm in high-glucose DMEM containing N2 (Invitrogen), B-27 (StemCell Technologies), 1 mM NAC, 1 mM sodium pyruvate, 2 mM L-glutamine, 20 ng/ml epidermal growth factor and 20 ng/ml basic fibroblast growth factor (both murine growth factors were from PeproTech). The total number of neurospheres at passage 0 (P0) was determined at 7 DIV by counting all neurospheres generated from each mouse brain. In subsequent passages, the total number of neurospheres was determined by counting all neurospheres generated in a 24-well dish seeded at 5×10^3 cells per well and extrapolating for the total number of dissociated cells.

Culture of NSPCs with microglia-conditioned medium

Microglia were plated at a density of 3×10^5 cells/cm^2 for 24h, followed by washing with PBS twice, and then addition of DMEM-F12 + GlutaMax medium for another 24 h. The microglial medium was collected and used as conditioned medium to culture NSPCs. Dissociated NSPCs were plated at 8×10^3 cells/cm^2 on poly-l-lysine-coated coverslips and maintained in the proliferation medium (above-mentioned microglia-conditioned medium containing N2, B-27, 1 mM NAC, 1 mM sodium pyruvate, 2 mM L-glutamine, 20 ng/ml epidermal growth factor and 20 ng/ml basic fibroblast growth factor) for 3 days or in the differentiation medium (above-mentioned microglia-conditioned medium containing N2, B-27, 1 mM NAC, 1 mM sodium pyruvate, 2 mM L-glutamine and 1% fetal bovine serum) for 7 days. Half of the medium was replaced with fresh medium every other day.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. You, Z. and Chen, H. (2021). Pharmacological intervention. Bio-protocol Preprint. bio-protocol.org/prep1433.
2. Zhang, J., Rong, P., Zhang, L., He, H., Zhou, T., Fan, Y., Mo, L., Zhao, Q., Han, Y., Li, S., Wang, Y., Yan, W., Chen, H. and You, Z.(2021). IL4-driven microglia modulate stress resilience through BDNF-dependent neurogenesis. Science Advances 7(12). DOI: 10.1126/sciadv.abb9888